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Functional Response of Fourth Instar Larvae and the Female *Serangium montazerii* Fursch (Coleoptera: Coccinellidae) to Different Densities of *Dialeurodes citri* (Ashmead) (Hemiptera: Aleyrodidae) Under Laboratory Conditions

Saiedeh Masoumeh FOTUKKIAII[1] Ahad SAHRAGARD [2*]

[1, 2] Department of Plant Protection, College of Agriculture, University of Guilan, Rasht, IRAN.
[2*] (Corresponding author) Department of Plant Protection, Faculty of Agriculture,
University of Guilan, Rasht, IRAN, Mobile: 0098 911 135 1540, fax: 0098 131 6690281
e-mail: sahra1330@yahoo.com; sahragard@guilan.ac.ir

ABSTRACT

Functional responses of fourth instar larvae (L4) and the female, *Serangium montazerii* Fursch to different densities (5, 10, 20, 30, 40, 60, 80 and 100 for females and a more 120 whiteflies for L4) of third and fourth instar nymphs of *Dialeurodes citri* were studied. These experiments were carried out in a growth chamber (25˚C, 65 ± 5% RH and a photoperiod of 16L: 8D h) on Thompson navel, *Citrus sinensis* cv. Thompson leaves. Each experiment was replicated five times. Using logistic regression, a type II functional response was determined for two stages of *S. montazerii*. The searching efficiency (a') and handling times (T_h) of the female adults and larvae by using Nonlinear least-square regression were estimated as 0.1614±0.0456 h^{-1}, 0.2540 ± 0.0587h^{-1} and 0.4641 ± 0.0377h^{-1}, 0.3715 ± 0.0174 h^{-1}, respectively**.** Maximum theoretical predations (T/T_h) estimated by the model of Rogers for larvae and females were 64.60 and 51.71, respectively. Although fourth instar larvae are more efficient than female, both life stages of *S. montazerii* may have the potential to be employed in biological control programmes against citrus white fly.

Key words: Biological control, handling times, lady beetle, predation rate, searching, efficiency.

INTRODUCTION

Dialeurodes citri (Ashmead) (Hemiptera: Aleyrodidae) is a polyphagous insect that infests evergreen and deciduous plants of 30 different families (Mound and Halsey, 1978). In Mediterranean countries, it mainly lives on *Citrus* spp., but its presence has also been noted on plants other than the family of Rutaceae. For example, Uygun *et al.* (1990) recorded 15 plant species other than citrus as hosts of *D. citri* in Mediterranean part of Turkey. It is one of the three economically important whiteflies on citrus in Mediterranean region (Rapisarda *et al*., 1996; Uygun *et al*., 1996). Citrus whitefly adults damage by feeding. Immature stages pierce the undersides of leaves with their mouthparts and suck plant sap. They excrete large quantities of honeydew that stimulates the sooty mould fungus growth. Heavy attacks may result in leaf-fall, especially during the summer. An additional form of damage is the external coating of fruits by sooty mould, which lowers their market value (Žaniĉ *et al*., 2001).

The lady beetle*, Serangium montazeri* Fürsch (Coleoptera: Coccinellidae) was recorded as a predator of the citrus whitefly, *D. citri* in Iran (Montazeri, 1994) and Turkey (Yigit *et al*., 2003). *S. montazerii* is also able successfully develop, survive, reproduce and feed upon *Bemisia tabaci* (Gennadius, 1889). Consequently, this ladybeetle seems to have a potential to be a biocontrol agent of the cotton whitefly, which could be employed in biological control programmes against this pest in glass-houses and open fields (Al-Zyoud, 2008).

One criterion to evaluate the efficiency of a predator is its feeding response to changes in prey density, or its functional response, defined as the number of prey that an individual predator kills as a function of prey density (Holling, 1966). The functional response may represent an increasing linear relationship (Type I), a decelerating curve (Type II), or a sigmoidal relationship (Type III) (Pervez and Omkar, 2005). A type II functional response with a decelerating predation rate has the potential to destabilize prey- predator population dynamics due to an inverse density-dependent mortality of the prey (Hassell, 1978). In contrast, the type III functional response, which incorporates density-dependent prey mortality, may stabilize the dynamics (Murdoch and Oaten, 1975). An investigation on the functional response of the ladybeetle, *Scymnus subvillosus* Goeze at different densities of the mealy plum aphid *Hyalopterus pruni*, Geoffroy showed that response of each larval stage, and adults matched Holling's type II functional response (Atlihan and Guldal, 2009). The functional responses of female and male, third and fourth instars larvae of *S. syriacus* Marseul to different densities of *A. spiraecola* Patch were found to be a type II functional response (Emami, 1996). This type of functional response has also been recorded for many different coccinellids, including: *Aphidecta obliterata* (L.) and *Adalia bipunctata* (L.) preying on *Elatobium abietinum* (Walker) (Timms *et al*., 2008), larvae and adults of *Propylea quatuordecimpunctata* (L.) preying on *Diuraphis noxia* (Mordvilko) (Messina and Hanks, 1998), *Coccinella undecimpunctata* (L.) preying on *A. fabae* and *Aleyrodes proletella* (L.) (Moura *et al*., 2006).

Since the knowledge on the functional response of *S. montazerii* is still lacking in the literature. Therefore, the functional responses of fourth instar larva and female of *S. montazerii* were studied under laboratory conditions.

MATERIALS AND METHODS

Predator and Prey cultures

The third and fourth instar nymphs of *D. citri* and the fourth instar larvae and females of *S. montazeri* were collected from a citrus orchard in Ramsar (Mazandaran province, North of Iran). All whiteflies and predators stocks were kept at 25±1˚C, 65 ± 5% RH and a photoperiod of 16:8 (L:D) h.

Functional response

To study the functional response of *S. montazerii*, individual adults and larvae of this predator were separately presented to different densities of third and fourth instar nymphs of *D. citri* (5, 10, 20, 30, 40, 60, 80 and 100 for females) and a more 120 whiteflies for L4 in Petri dishes (9 x 1 cm). In order to provide required densities of third and fourth instar nymphs of prey, excessive nymphs on the host plant leaves were removed by a scalpel. Individual females and fourth instar larvae of *S. montazerii* were starved for 24 h. Each experiment was carried out under laboratory conditions as above and replicated five times. After 24 h, predators were removed from the Petri dishes and the number of whitefly nymphs eaten by each female and larva was recorded.

Data analysis

The type of the functional response was determined by logistic regression analysis [SAS/STAT, CATMOD procedure (SAS version 9.1)] of the proportion of prey consumed (Ne) in relation to initial prey density (N_0) (Trexler and Travis, 1993). In the logistic regression a cubic model was used (Juliano, 1993):

$$N_e/N_0=\exp\ (P_0+P_1N_0+P_2N_0^2+P_3N_0^{3)}/\ (1+\exp\ (P_0+P_1N_0+P^2N_0^{\ 2}+P_3N_0^{\ 3})) \quad (1)$$

where P_0, P_1, P_2 and P_3 are the parameters to be estimated. If the linear parameter P_1 is negative, a type II functional response is evident, whereas a positive linear parameter indicates density-dependent predation and thus a type III functional response (Juliano, 1993). Once the type of functional response was determined, both the disc equation (Holling, 1959b) (2) and the random attack equation (Royama 1971; Rogers 1972) (3) were used to estimate handling time (T_h) and searching efficiency or attack rate (*a'*). For the type II response, the equations are as follows:

$$N_a = (a'\ T\ N_0)/\ (1+ a'T_hN_0) \qquad (2)$$

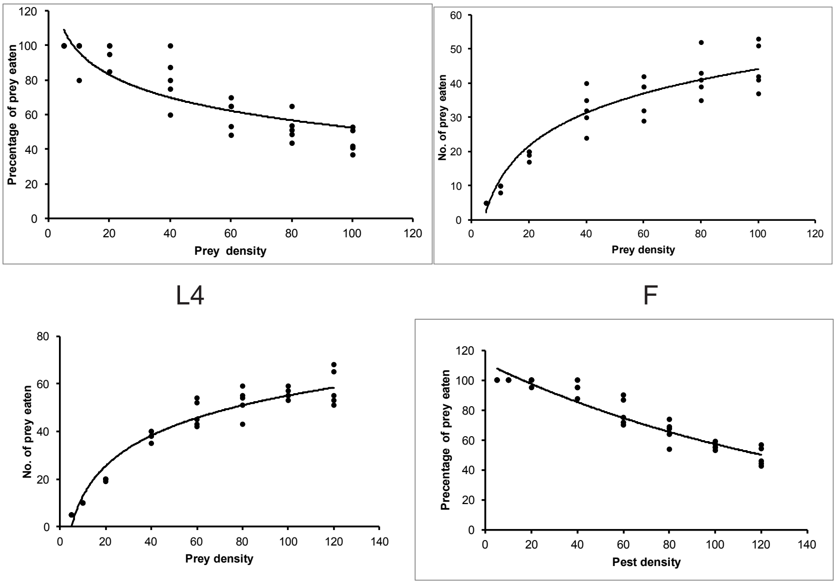
$$N_a = N_0\,[1\text{-}\exp\ (a'\ (T_h\,N_0 - T))] \qquad (3)$$

Where N_a is the number of prey eaten, N_0 is the number of prey offered, T is the total time available for the predator, *a'* is the searching efficiency, and T_h is the handling time.

Statistical analysis of the functional response was performed using the SAS software (SAS Institute, 2001). A nonlinear regression was used (the least square technique with DUD initialization) to estimate predator handling time and searching efficiency.

RESULTS

The logistic regression for both fourth instar larvae and female predator had a significant (P= 0.0028, 0.0006) linear parameter $P_1 < 0$ (Table 1) and the proportion of prey consumed by both stages of predator declined with increasing prey density (Fig. 1). This suggests that they both exhibited a Type II functional response.



L4 F

L4 F

Fig 1. Functional response of *Serangium montazerii* on different densities of *Dialeurodes citri*: fourth larval instar (L4) and female (F). Symbols and lines represent the observed and predicted values, respectively.

Table 1. Maximum likelihood estimates from logistic regression of proportion of prey eaten as a function of initial prey densities by female and fourth larval instar of *Serangium montazerii*.

	Parameter	Estimate	SE	X^2	*P*
Female	Constant	7.2292	1.4089	26.33	<.0001
	Linear	-0.2402	0.0702	11.71	0.0006
	Quadratic	0.00281	0.00110	6.47	0.0110
	Cubic	-0.00001	5.46E-6	4.41	0.0357
4th larval instar	Constant	9.0587	1.9227	22.20	<.0001
	Linear	-0.2235	0.0747	8.96	0.0028
	Quadratic	0.00197	0.000926	4.52	0.0335
	Cubic	-6.16E-6	3.664E-6	2.82	0.0930

The values of coefficient of determination (R^2) and standard errors of the estimated parameters indicated that both equations (disc and random predator equations) adequately described the functional response of larvae (R^2= 0.9898, 0.9910) and females (R^2= 0.9802, 0.9807) of *S. montazerii*, respectively. However, Rogers random attack equation yielded lower residual sum squares (649.4 and 545.0 for the fourth instar larva and female, respectively) indicating a better fit than did the Holling disc equation (691.5 and 664.7).

Their handling times (T_h) and coefficient of attack rates (*a*') (estimated by Holling disc equation and Rogers random attack equation) show that larvae had a shorter handling time and higher searching efficiency than the females (Table 2).

The estimated maximum rates of predation (T/T_h) using the random attack equation for the fourth instar larvae and females were 64.60 and 51.71, respectively.

Table 2. Coefficient of attack rate (a') and handling time (Th) [estimated by Holling disc equation and Rogers random attack equation] for female and fourth larval instar (L4) of *Serangium montazerii* fed on third and fourth instar nymphs of *Dialeurodes citri*.

Model	Stage	Parameter	Estimate	SE	95% CI		T/T_h	R^2
					Lower	Upper		
Holling	Female	a'	0.0553	0.00646	0.0422	0.0685	68.87	0.9802
		T_h	0.3485	0.0330	0.2814	0.4156		
	L4	a'	0.0607	0.00489	0.0706	0.0508	92.059	0. 9898
		T_h	0.2607	0.0172	0.2995	0.2955		
Rogers	Female	a'	0.1614	0.0456	0.0686	0.2542	51.71	0.9807
		T_h	0.4641	0.0377	0.3874	0.5408		
	L4	a'	0.2540	0.0587	0.1351	0.3729	64.60	0. 9910
		T_h	0.3715	0.0174	0.3362	0.4067		

DISCUSSION

The functional response of a natural enemy offers a good conceptual framework for understanding the action of biological control agents in inundative releases (Waage and Greathead, 1988). The estimated maximum numbers of whiteflies attacked by fourth instar larvae was higher than female. Although other larval instars of the predator may have important role on prey population suppression, our results showed that female and fourth larval instar are the stages of *S. montazerii* that are effective predators on citrus whitefly. Similar results were found on *Hippodamia variegata* Goeze preying on *A. fabae* (Scolpoli) (Farhadi *et al*., 2010).

A type II functional response was displayed on *D. citri* by two stages of *S. montazerii*. This type of functional response is the most common functional response in insects and has been reported for many different coccinellids, including: *C. septempunctata* and *H. variegata* preying on third and fourth nymphal instars of *B. tabaci* (Gahari *et al*., 2003), all stages of *H. variegata* (G.) on *A. fabae* (Farhadi *et al*., 2010), male adults of *Cheilomenes sulfurea* Grote preying on *A. fabae* (Hodek *et al*., 1984), adult females of *Cheilomenes sexmaculata* (Fabricius), *Propylea dissecta* (Mulsant), and *C. transversalis* (Fabricius) preying on *A. craccivora* (Koch) or *Myzus persicae* (Sulzer) (Pervez and Omkar, 2005).

Functional response experiments are usually conducted in controlled conditions. Clearly, in nature, the predator will encounter uncontrolled and highly variable conditions. Changes in temperature, humidity, and other aspects of weather could radically change the functional responses of predators to variation in prey density. It is difficult to predict a priori, whether such climatic effects will increase or decrease the number of prey eaten at each density (Farhadi *et al*., 2010).

The handling time (T_h), is a good indicator of the predation rate (Atlihan and Guldal, 2009). Its value, for larvae was lower than females in this study. Farhadi *et al*. (2010), in contrast, found no significant differences between handling times of females and fourth instar larvae of *H. variegata* fed on *A. fabae*. The maximum theoretical predation rate (T/T_h) of larvae was greater than female. The values of searching efficiency estimated by both Holling and Rogers models for larvae were also greater than females. According to Nordlund and Morrison (1990), the handling time affects the type of functional response, that if it is shorter, the faster the curve reaches the asymptote, furthermore handling time can influence other components such as attack rate and searching efficiency (Beddington *et al*., 1976).

It is necessary to study the functional responses of other larval stages and even male insects in order to draw a general conclusion on the efficacy of this predator. However, it can be concluded here that fourth instar larvae are more efficient than female *S. montazerii* and both may have the potential to be employed in biological control programmes against citrus whitefly.

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